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Metabolic effect of short term administration of *Hoodia gordonii*, an herbal appetite suppressant

S. Jain, S.N. Singh *

Nutrition Division, Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi, 110054, India

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ABSTRACT

Hoodia gordonii (family: Apocynaceae) is used traditionally by the Khoi-San tribes to control hunger. It has become extremely popular and has triggered commercial interest due to its appetite suppressant property. The present study was undertaken to investigate the appetite regulatory mechanism and associated metabolic changes induced by the herb. Effect of organic solvent extract of *H. gordonii* on food intake and body weight of male *Sprague Dawley* rats was monitored at three different doses 50, 100 and 150 mg/kg body weight, given orally for five days. Subsequently, the dose of 100 mg/kg body weight was selected for further studies on the regulatory hormones and biochemical variables. Dose-dependent reduction in food intake (12–26%) was observed at a dose of 100 and 150 mg/kg body weight ($p<0.05$). Appetite suppression persisted for 6 h and food intake was restored within 24 h after stopping of the treatment. There was an increase in liver glycogen stores, activity of mitochondrial CPT-1 and thyroid hormones in treated animals. The circulating levels of NPY and IGF-1 were decreased with marginal increase in leptin and CCK, in case of treated rats. There was no change in blood glucose and insulin levels were not affected significantly. The hormonal and metabolic changes due to treatment with the *H. gordonii* extract may be responsible for its anorectic activity.

1. Introduction

There has been a global increase in the use of herbal products for health care (Hermann and von Richter, 2012). Traditional systems of medicine rely on herbs for their medicinal properties (Arun and Nalini, 2002; Caili et al., 2006). *Hoodia gordonii*, is a popular supplement and one of the most widely consumed appetite suppressant of natural origin. It is a succulent plant that belongs to the family Apocynaceae and grows throughout South Africa and Namibia (Brunys, 2005). *Hoodia* with the name 'Xhoba' have been in use by the indigenous Khoi-San tribes of the Kalahari during their long hunting trips to curb hunger or during periods of famine. Further investigation by the Council for Scientific and Industrial Research (CSIR, South Africa) about the genus *Hoodia* led to the finding that out of the 13 reported species only *H. gordonii* and *H. pilifera* exert anorectic activity (Avula et al., 2006). Initial studies demonstrated a decrease in food intake and body weight in obese and lean rats (Tulp et al., 2001, 2002). It is reported that *H. gordonii* is a rich source of pregnane, oxypregnane and steroidal glycosides (Shukla et al., 2009). Experiments performed by MacLean and Luo (2004) demonstrated that a specific steroidal glycoside, P57 when administered by i.c.v injection increases the amount of ATP in the hypothalamus, which stimulates satiety and is indicative of a central mechanism of action. However, a pharmacokinetic study revealed that oral administration of *Hoodia* extract, does not cause an increase in P57 in brain (Madgula et al., 2010). The precise mechanism of its action has not been elucidated. Limited human clinical trials with *H. gordonii* have been done. An observational pilot research study was performed on obese participants and effects on body weight and appetite were assessed (Holt and Taylor, 2006). A specific product called Hoodia Supreme was administered twice daily in form of capsule that contained 400 mg of pure *H. gordonii* per capsule. Reduction in appetite (500–1000 calories per day) and weight loss (2–15 lb) were reported in all subjects over the duration of 4 weeks (Holt and Taylor, 2006). In a double blind, placebo controlled study, a group of obese free- feeding volunteers were treated with *H. gordonii*. A reduction of food intake up to 1000 calories per day (approx.) and body weight loss of about 2 kg along with decrease in blood glucose and triglycerides was observed (Glasl, 2009; Holt and Taylor, 2006).

A purified solvent extract, HgPE (*H. gordonii* purified extract) containing 79.3% w/w steroid glycosides was formulated as a flavoured yoghurt drink (Blom et al., 2011). This HgPE caused a significant dose dependent reduction in food intake and body weight of rats when repeatedly administered for 13 weeks (Blom et al., 2011). This HgPE extract was also evaluated on healthy overweight women at dose of 1110 mg HgPE twice/day, 1 h before breakfast and dinner for a period of 15 days. The placebo group was given a yoghurt drink. The mean effects on *ad libitum* energy intakes and body weights did not differ significantly between the treated and placebo groups. Inspite of the enormous commercial interest in *H. gordonii* supplements, there is insufficient published scientific data on regulation of appetite. Some

* Corresponding author. Tel.: +91 1123883315.
E-mail address: nutrition-dipas@hotmail.com (S.N. Singh).

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researchers have confirmed the appetite suppressing property of *Hoodia* species but there are no reports on its effect on the appetite regulatory peptides. Based on this, the study was undertaken to further investigate the acclaimed anorectic activity of *H. gordonii,* its possible effects on energy metabolism and other biochemical changes using young adult *Sprague Dawley* rats as an experimental model.

2. Materials and methods

2.1. Plant material

Authenticated dry powder of (cultivated) *H. gordonii* was a gift from M/S Farm Vredelus PTY LTD, Farm Douglas, Namibia. Certificate of Analysis along with the LCMS chromatogram for *H. gordonii* were also provided by them (LC/MS analysis was done at University of Stellenbosch, South Africa).

2.1.1. Preparation of extract

Organic solvent extract was prepared using a mixture of dichloromethane-methanol (1:1) (Corley and Miller, 2009). Dry powder was weighed (20 g) and mixed with a 100 ml solvent mixture and kept overnight for extraction. The organic layer was separated from raw plant material by centrifugation at 3000 ×*g* for 15 min at room temperature. Extraction was repeated twice under identical conditions, supernatants were combined and dried at 40 °C. The yield of the extract was 8.0% of the dry powder supplied by the manufacturer.

2.2. Animals, treatment, monitoring of food intake and body weight

Male *Sprague Dawley* rats, weighing 200–250 g, bred and reared in the Experimental Animal Facility of the Institute were used in the study. Animals were maintained at a temperature of 22° ± 1 °C and a humidity of 55–60% in light-controlled room (lights on at 6:30 h, lights off at 18:30 h). Rats were habituated to a restricted day-time feeding regimen two weeks prior to the experimentation by providing them food only for 6 h during the light phase for monitoring of food intake. Rats were provided commercial rodent diet supplied by M/S Golden feed Pvt Ltd., Delhi and water *ad libitum*. All procedures and protocols used in the present study were approved by the Animal Care and Use Committee of the Institute and followed the guidelines documented in the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The animals were randomly divided into control and experimental groups prior to the beginning of the experiments.

Initially six groups of animals (n = 6 in each) were treated with crude alcoholic extract at doses of 50, 100 and 150 mg/kg body weight orally using a rodent feeding needle once daily for five days. The respective control groups for each treatment group received equal volume of tap water using a rodent feeding needle. The extract was given in the morning every day for five days, at the time of providing food and water to the animals. The minimum effective dose of 100 mg/kg body weight was selected subsequently for further biochemical experiments. Food was weighed on a digital balance every 2 h i.e. at 2 h, 4 h and 6 h to see if the extract has a continuous or progressive anorectic action. Food intake was monitored additionally for three days (over an eight day period) to find out any residual effect after stopping the treatment. This was performed on a separate batch of rats comprising of two groups, control and treated (n = 6 in each). Changes in body weight were monitored daily post treatment.

2.3. Sample collection for biochemical analysis

After completion of the five day treatment, rats were fasted overnight, anaesthetized and sacrificed. Blood plasma was collected by centrifugation at 1000 ×*g* for 10 min at 4 °C. The weight of the organs — liver, spleen, kidney, brain was recorded along with gastrocnemius muscle and epididymal fat tissue. For glycogen estimation the weighed portions of liver were dissolved in 30% KOH immediately after removal, precipitated with 95% ethanol in the presence of sodium sulphate. Ten percent liver and brain homogenates (w/v) were prepared in 150 mm KCl using Polytron homogeniser and were centrifuged at 3000 ×*g* for 15 min at 4 °C. The supernatants were divided into aliquots and frozen at −80 °C until assayed. Liver mitochondria were separated by differential centrifugation at 12,000 ×*g* for 30 min at 4 °C and stored at −80 °C for CPT-1 activity analysis.

In a separate experiment to study the effect on blood glucose levels, samples were collected from the retro-orbital plexus at 1 h, 2 h and 4 h after giving an oral dose of 100 mg/kg body weight (n = 6).

2.4. Biochemical estimations

Blood glucose was measured using glucose oxidase–peroxidase method. Tissue glycogen was estimated using the method of Montgomery (1957). CPT-1 (Carnitine Palmitoyl Transferase-1) activity was assayed using the method of Halperin and Pande (1979). Mitochondrial protein content was measured by the method of Lowry et al. (1951). Na^+/K^+ATPase activity was measured in the brain homogenate using the method of Esmann (1988). Radio immunologic assay (RIA) kits from Phoenix Pharmaceuticals, Burlingame, CA were used for detection of plasma ghrelin, neuropeptide Y (NPY) and cholecystokinin (CCK) levels. Plasma leptin and adiponectin concentrations were measured with a commercially available rat ELISA kit from Ray Biotech, Inc., USA. Plasma insulin concentrations were determined using a direct ELISA kit bought from Mercodia, Uppsala, Sweden. Plasma samples were extracted using ethyl ether for corticosterone estimation and estimated using an ELISA kit (Neogen Corporation, USA). Plasma levels of insulin-like growth factor 1 (IGF-1) were analyzed with an EIA kit from Mediagnost, Germany. AMP kinase activity, levels of tri-iodothyronine (T3), thyroxine (T4) and 5-Hydroxytryptamine (5-HT) in brain homogenates and plasma were measured using kit from Cusabio biotech Co., Ltd.

2.5. Statistical analysis

All the data are presented as mean ± SD and statistically analyzed using unpaired Student's *t*-test. The p value<0. 05 was considered significant change.

3. Results and discussion

3.1. Effect on food intake, body weight and organ weights

H. gordonii crude extract at oral dose of 100 mg/kg and 150 mg/kg produced a dose-dependent decrease in food intake over the 6 h period on all the five days. There was no change in food intake at a lower dose of 50 mg/kg body weight (Table 1). There was a decrease in food intake by 17.5% (12.4%–22.3%) and 22.8% (18.4%–26.7%) respectively at dosage of 100 and 150 mg/kg body weight ($p<0.05$) (Table 1). In the experiment carried out to monitor food intake at different time points a reduction in food intake by 15% was observed at 2 h following administration and remained up to the sixth hour (Fig. 1a). The food intake returned to the levels of untreated group from day 6 to day 8 after the 100 mg/kg body weight dose was discontinued on day 5 (Fig. 1b). This indicates that the extract has a transient action and not a sustained effect. The body weight was not affected in the treated animals (3.8%) compared to control (3.6%) at the dose of 50 mg/kg body weight (Fig. 1c). Similarly, at dose of 100 mg/kg body weight there was no significant effect on body weight in treated animals (3.7%) compared to control (4%) (Fig. 1d). At 150 mg the rate of body weight gain in treated rats was 1.8% as compared to 6% in control indicating a reduction in weight gain due to treatment (Fig. 1e). No significant differences in the weights

Table 1
Effect of crude *H. gordonii* extract on food intake.

Oral treatment (mg/kg body weight)		Day 1	Day 2	Day 3	Day 4	Day 5	Mean ± SD
50	C (g)	96.0	100.0	95.0	96.0	99.0	97.2 ± 2.4
	T (g)	97.0	101.0	94.9	96.0	100.0	97.7 ± 2
	Diff (g)	+1	+1	−0.1	0	+1	0.5 ± 0.6
	Diff (%)	1.04	1	2.7	0	1	
100	C (g)	97	101	100	95	97	98.0 ± 2.4
	T (g)	85	84	81.3	78.5	75.4	80.8 ± 4.0
	Diff (g)	−12	−17	−18.7	−16.5	−21.6	17.2 ± 3.5*
	Diff (%)	12.4	16.8	18.7	17.4	22.3	
150	C (g)	97	99	95	95.5	97.6	97.8 ± 1.4
	T (g)	74.5	78	77.5	72	71.5	74.7 ± 2.7
	Diff (g)	−22.5	−21	−17.5	−23.5	−26.1	22.1 ± 2.8*
	Diff (%)	23.2	21.2	18.4	24.6	26.7	

Values are mean ± SD (n = 6), C: Control, T: Treated, *$p<0.05$; + denotes an increase and − denotes a decrease in food intake in comparison to control.

of vital organs and epididymal fat tissues were found between the groups (data not shown).

The decrease in food intake and reduction in weight gain in the treated groups in the present study indicates appetite suppression in rats and this observation is similar to earlier reports (van Heerden et al., 2007; MacLean and Luo, 2004; Tulp et al., 2001). However, the form of the herb used i.e. pure glycoside or a formulated product, the dosage and the extent to which food consumption was suppressed varied. The recovery in food intake after stopping the treatment is also reported in an earlier study by van Heerden et al. (2007). Holt (2005) quotes *H. gordonii* is not a 'magic bullet' for overweight problems but imperatively modifies food behavior and thereby leads to the controlled energy intake. Human trial with *H. gordonii* showed reductions in food intake and body weight from baseline but with no significant difference between the experimental and placebo groups (Blom et al., 2011). This could be due to the complex interactions between the biological mechanisms of appetite and environmental challenges that determine drive to eat.

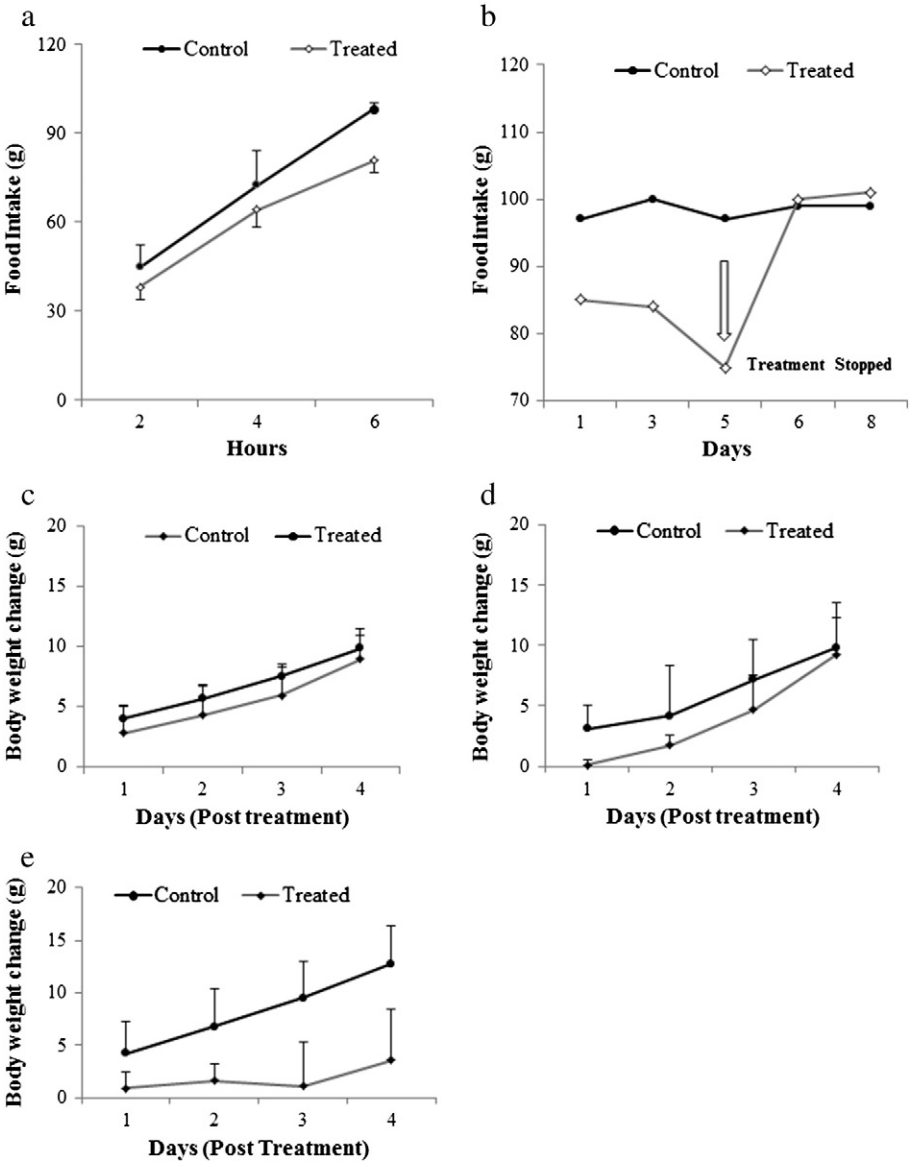

Fig. 1. (a) Effect of *H. gordonii* treatment on food intake at specific time points for 6 h at dose 100 mg/kg body weight (n = 6). (b) Recovery in food intake after stopping treatment with 100 mg/kg body weight (n = 6), combined food intake of 6 rats. (c) Changes in body weight of rats following *H. gordonii* administration at dose of 50 mg/kg body weight (n = 6). (d) Changes in body weight of rats following *H. gordonii* administration at dose of 100 mg/kg body weight (n = 6). (e) Changes in body weight of rats following *H. gordonii* administration at dose of 150 mg/kg body weight (n = 6).

3.2. Effect on blood glucose and liver glycogen

The blood glucose levels were not affected by treatment at 1 h, 2 h and 4 h post intervention (Fig. 2). However, there was a significant increase ($p<0.01$) in the hepatic glycogen content (control: 0.46 ± 0.75 mg/g wet tissue, HG: 1.77 ± 0.72 mg/g wet tissue). Although the herb is postulated to alter appetite through the CNS, it may also have a peripheral action (MacLean and Luo, 2004). The increase in liver glycogen is probably related to a change in sugar metabolism through the glycosides present in *Hoodia* (Holt, 2006). The blood glucose concentration signals the induction of hunger sensation in rats (Poothullil, 1992). It has been suggested that the effects of *Hoodia* on blood glucose and insulin are not direct, and may occur only when on a calorie reduced diet or in combination with cinnamon and anti-hyperglycaemic compounds to treat Syndrome X (Holt, 2006). Increased glycogen production leads to an early feeling of fullness. This is achieved by stimulating the glucoreceptors that sends satiety signals to the brain via the vagus nerve (Anderson, 1994).

3.3. Effect on enzyme activities

The gastrointestinal tract and the adipose tissue both send signals to the brain and regulate the energy homeostasis (Stanley et al., 2005). Since the steroidal glycoside, P57 present in *H. gordonii* resembles the structure of cardiac glycosides (MacLean and Luo, 2004; Tucci, 2010) the activity of Na^+/K^+ATPase was measured in whole brain and no change was observed (control: 0.018 ± 0.01 mmol/mg protein/min, HG: 0.019 ± 0.02 mmol/mg protein/min). CPT-1 activity in the isolated liver mitochondria was found to be significantly increased in the treated group (control: 10.81 ± 4.50 μmol/min/mg protein, HG: 16.73 ± 6.54 μmol/min/mg protein, $p<0.05$) while the AMPK activity measured in crude liver homogenates was not changed with HG supplementation (control: 314 ± 54 pg/mg protein, HG: 292.97 ± 9.18 pg/mg protein, $p<0.05$). The significantly increased activity of CPT-1, an enzyme of the fatty acid oxidation, may be in part attributed to the raised adiponectin in the present study, this may affect lipid metabolism and enhances fatty acid oxidation (Xu et al., 2003). However, it appears that adiponectin did not exert its effect peripherally to stimulate the liver AMP kinase activity, thereby not increasing food intake (Kubota et al., 2007). Depleting Adenosine triphosphate (ATP) or increasing Adenosine monophosphate (AMP) levels activates AMPK (Shetty et al., 2009). This reinforces the observation, as a no significant change in the AMPK activity could be due to no ATP depletion resulting in a feeling of satiety (MacLean and Luo, 2004).

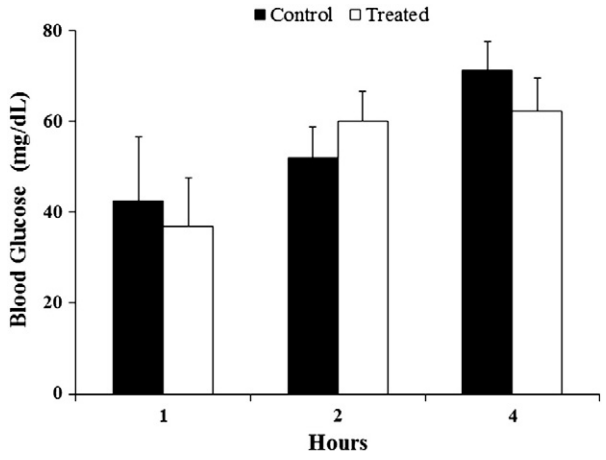

Fig. 2. Effect on blood glucose levels measured at 1 h, 2 h and 4 h after *H. gordonii* supplementation at 100 mg/kg body weight ($n=6$), no food restriction during the experiment was done.

3.4. Effect on appetite regulatory peptides

Plant extracts usually target several systems of the body as majority of them are not single compounds but a mixture of different molecules. Also, since some cellular receptors tend to be widely distributed, a single molecule can have several metabolic effects (Tucci, 2010). Food intake and the appetite hormone systems like leptin, ghrelin and IGF-1 closely affect the nutritional status.

Insulin regulates glucose homeostasis by increasing glucose uptake, and the fall of blood glucose is a stimulus for increased appetite. The insulin levels did not show any significant change in the present study. Insulin is known to increase the production of IGF-1 (Butler and LeRoith, 2001). Low IGF-1 levels are seen in fasting and are increased in response to high calorie intakes (Sridhar and Goodwin, 2009). The decline in the plasma IGF-1 levels ($p<0.05$) in the present study may be in response to the lower calorie intake.

A decline in ghrelin levels has an appetite lowering effect while leptin decline has appetite stimulating effects. In the present study an increase in leptin and concomitant suppression in ghrelin levels, though values were statistically not significant. Plasma ghrelin concentrations are known to correlate with calories ingested (Cummings et al., 2001). The NPY/AgRP neurons play an important role as they mediate the effect of ghrelin (Cowley et al., 2003) and since leptin receptors are present on these neurons, leptin inhibits NPY (Baskin et al., 1999). The significant decrease in plasma NPY ($p<0.05$) and leptin levels show a similar association in the present study. Apart from neuropeptides, the role of monoamines in eating has also been observed. The 5-HT is a suggestive satiety agent (Fernstrom and Fernstrom, 2001). No significant effect of *H. gordonii* on serotonin levels were observed. Therefore possibility of serotonin reuptake inhibition remains unidentified and needs further study. Both serotonin and corticosterone in rodents have the highest peak during the active period of the diurnal cycle. In the present study circulating levels of corticosterone, those of the light period, were found to be significantly decreased ($p<0.05$) in the treated group. Glucocorticoids stimulate food intake by promoting NPY functions (Woods et al., 2004). A decline in both of these peptides in this study may also have a role in appetite suppression. A marginal increase in CCK secretion was also noted. CCK is known to be a peripheral gastrointestinal satiety signal. Recent research has proposed that the bitter taste of *H. gordonii* may lead to secretion of CCK and influence appetite control (Le Nevé et al., 2010). Additionally the appetite and metabolic rate are controlled through the peripheral effects of thyroid hormone that have clinical significance on energy intake. These effects were thought to be peripheral, but only recently it's been known to act centrally as well (Amin et al., 2011). T3 and T4 displayed a significant rise ($p<0.05$) after the supplementation. This could lead to an assumption of elevated basal energy expenditure arising from increased fuel metabolism (Pucci et al., 2000). It could have a role in lipolysis, though its function in lipid regulation is complicated (Mariash, 2003). Besides, thyroid hormones promotes carbohydrate metabolism and maintains glucose homeostasis (Chidakel et al., 2005). The levels of glucose and glycogen in the treated group may be mediated to a certain extent by the increased thyroid hormones (Table 2).

In the present study changes in levels of appetite regulatory peptides in response to *H. gordonii* supplementation *in vivo* may be responsible for decreased food intake. However, it is difficult to pin point any one molecule responsible for the action as appetite regulation is a complex phenomenon and plant extract used may have several compounds other than P57. The short term administration of the crude extract generates adequate evidence of it being an appetite suppressant. This implies that if given for a longer period it may exert a pronounced effect. Traditional belief that herbs are safer than synthetic compounds is one of the classical arguments, which is yet to be proven. The extensive

Table 2
Effect of crude *H. gordonii* extract supplementation (100 mg/kg body weight) for five days on different hormones in plasma.

Appetite regulatory peptides	Experimental groups	
	Control	Treated
Insulin (ng/ml)	0.36 ± 0.05	0.30 ± 0.03
Insulin-like growth factor 1 (ng/ml)	477.50 ± 136.50	371.29 ± 140.12*
Leptin (pg/ml)	839.59 ± 395.16	956.01 ± 549.86
Ghrelin (pg/ml)	1204.58 ± 141.06	1159.00 ± 53.63
CCK (pg/ml)	281.18 ± 16.93	307.83 ± 18.22
Adiponectin (ng/ml)	82.40 ± 56.98	101.81 ± 87.77
Brain serotonin (ng/ml)	1.19 ± 0.14	1.09 ± 0.29
Plasma serotonin (ng/ml)	64.62 ± 26.30	78.10 ± 31.16
Corticosterone (ng/ml)	67.67 ± 11.23	56.70 ± 12.38*
Neuropeptide Y (pg/ml)	1124.41 ± 535.31	605.41 ± 397.81*
Tri-iodothyronine (ng/ml)	0.94 ± 0.16	1.47 ± 0.23*
Thyroxine (ng/ml)	35.75 ± 3.75	40.08 ± 2.57*

Expressed as mean ± SD (n = 6), *p < 0.05 in comparison with control.

effects on carbohydrate and fat metabolic enzymes and substrates remain undetermined. Present investigation on experimental animals needs further studies on humans since the feeding behaviour of humans and rodents are different.

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Conflict of interest

The authors declare that they have no conflict of interest.

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